European Journal of Medical and Health Sciences
www.ejmed.org

Term Pregnancy Woman with Human Immunodeficiency Virus Infection and Antiretroviral Therapy as Risk Factor of High Caspase-3 Expression in Placenta

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ABSTRACT

Aim: **To determine whether term pregnant women with human immunodeficiency virus infection who received antiretroviral therapy have a high Caspase-3 expression in the placenta.**

Methods: **This cross-sectional analytical study was conducted at Sanglah General Hospital, Denpasar, and affiliated hospitals. Subjects were termed pregnant women with HIV (+) who received a minimum of six months of antiretroviral therapy as a risk group and pregnant women with HIV (-) as a non-risk group. Caspase-3 expression was assessed by immunohistochemical examination of placental tissue. The cut-off value for caspase-3 levels was determined by constructing a receiver operating characteristics (ROC) curve. The difference in proportion was assessed by the chi-square test, and the prevalence ratio (PR) was reported. The significance of this study was $p<0.05$.**

Results: **Each 20 pregnant women were included in the risk and no-risk groups. There was no difference in age, gestational age, parity, and body mass index (BMI) between the two groups. The mean expression of caspase-3 was significantly higher HIV (+) group than in the HIV (-) group (162.62 vs. 105.83; p = 0.000). The selected cut-off value of Caspase-3 was 127.05, with a sensitivity of 75% and a specificity of 75%. After classification, a significantly higher proportion of Caspase-3 expression was found in the HIV (+) group than in the HIV (-) group (75% vs. 25%; p=0.002). Pregnant women with HIV (+) and receiving antiretroviral therapy for a minimum of six months had a three times higher chance of having a high Caspase-3 prevalence than pregnant women who were not (RP=3.0; 95% CI = 1.348 – 6.678).**

Conclusion: **Pregnant women with HIV infection who received antiretroviral therapy have a high Caspase-3 expression in the placenta.**

Keywords: Antiretroviral therapy, Caspase-3, human immunodeficiency virus, term pregnancy.

Submitted : April 9, 2022

Published : May 17, 2022

ISSN: 2593-8339

DOI: 10.24018/ejmed.2022.4.3.1324

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I. Introduction

HIV infection can happen to everyone, however enough trouble _ get attention is Mother pregnant with HIV infection, p this because risk transmission that occurs by vertical from Mother to unborn baby. Baby born from Mother with HIV infection tends to experience disturbance growth, birth premature in- room care intensive older neonates and development poor immunity.

HIV is a global health problem, including in Indonesia. In 2016, there were 34.5 million adult patients living with HIV in the world, and 15.3 million (44%) of them were women of reproductive age. In Indonesia, HIV cases tend to increase every year. The Indonesian Ministry of Health reported that cumulative HIV cases in Indonesia from April 1, 1987 to September 30, 2014 were 150,296 people, while for cases of Acquired Immune Deficiency Syndrome (AIDS) there were 55,799 people. The number of HIV infections in Bali until December 2017 reached 1,739 cases and AIDS reached 734 cases [1], [2]. PPIA data shows that the proportion of HIV positive in pregnant women has exceeded 1% (1.35%). This

figure is consistent with the results of the IBBS for the general population (2.3%) for five years (2011-2015). The ratio of HIV cases in men and women is 1:1,2-1.5, which means that women are more affected by HIV infection than men [3].

Various efforts have been made to reduce the rate of mother-to-child transmission in Indonesia, one of which is the provision of Antiretroviral Therapy (ART). Giving ART to women before, during pregnancy and breastfeeding, can prevent the transmission of disease from mother to child. Although antiretroviral therapy can reduce the risk of mother-to-child transmission, it can simultaneously increase the risk, especially premature birth, fetal growth restriction and preeclampsia, whose mechanisms are not fully understood [4].

As is enhancement outside bad pregnancy in the baby born from HIV mother after get ART treatment, so that need known how effect from ART drugs especially group Non-Nucleotide Reverse Transcriptase Inhibitors (NRTIs) can trigger happening depletion mitochondrial DNA (mtDNA) so that causes apoptosis in the placenta. Placental apoptosis in pregnant women with HIV can be triggered directly by viral particles or as a result of ART treatment. There are two main pathways for apoptosis, based on the involvement of Caspase; Caspase-dependent and Caspase-independent apoptosis. Caspase-dependent apoptosis is a classic pathway of programmed cell death, typically involving the aspartate-specific proteases Caspase-8, Caspase-9, Caspase-12, Caspase-7 and Caspase-3. Pro-apoptotic Caspase-3 is a caspase that is primarily activated during apoptosis and is triggered by receptors for mediators of the extrinsic and intrinsic mitochondrial pathways. While in Caspase-independent apoptosis, Caspase is not involved in the apoptotic process.

In addition to a decrease in mtDNA levels, immunohistochemical analysis found the active expression of Caspase-3 in fetuses with IUGR, significantly higher than in normal pregnancies. This study concluded that the pro-apoptotic expression of Caspase-3 in the placenta is one of the reasons for IUGR [5].

ART group non-nucleotide reverse transcriptase inhibitors (NRTIs) cause a number of effects especially DNA polymerase beta and gamma as well as in more influence small in DNA polymerase alpha. On cells placenta occur increase in mitochondrial mass in significant amount along with increase age pregnancy. Growth placenta occur enough fast for balance enhancement need fetal nutrition and oxygen. Placenta is membrane important that bridges exchange nutrition and products exile Among maternal and fetal circulation. Existence Abnormalities in structure and function composer circulation this is pattern dominant from injury placenta [6]. Angiogenic process starting in the second trimester trigger happening remodeling architecture vessels blood placenta for make it easy happening Genre blood to surface placenta so that make it easy happening displacement nutrients and oxygen. Happening disturbance remodeling spiral arteries due to failure invasion later trophoblast trigger drop Genre blood to placenta will causing happening ischemia placenta. Administration of ART in pregnancy with HIV can trigger hypertension nor preeclampsia during pregnancy. Although change underlying immunological happening preeclampsia in women pregnant with HIV no fully understood, by general known consequence an inflammatory process. Growth fetus obstructed (GFO) is distinguished Becomes type asymmetrical and symmetrical. If occur abnormality circulation uteroplacental consequence from development abnormal placenta, supply oxygen, input nutrition and production results metabolic to be abnormal.

A number of studies has found incident more preterm delivery higher in women on ART if compared to with girl who doesn't give ART. Study confirm that HIV infection increase 2.5 times the risk preterm delivery. HIV infection gives enhancement 2.1 times risk for spontaneous preterm delivery, and a 3.2-fold risk for iatrogenic preterm delivery7.Mechanism Immunology is also associated with complications obstetrics consequence ART drugs. Normal pregnancy happens enhancement Th2 lymphocytes (IL-10) and suppression of Th1 cytokines (IL-2). ART drugs cause shift from balance Th2 to Th1 cytokines. Change immunological this could prevent development HIV disease but caused preterm delivery.

II. Discussion

This study uses the Saphiro Wilk normality test with the results of the analysis showing that the age and gestational age data in both groups not normally distributed ($p < 0.05$), then the characteristics of the sample in this study were analyzed using the Mann-Whitney test (data available in the appendix). HIV patients on ART 6 months were the risk factor group and patients without HIV were the no risk group. The basic characteristics of the research sample can be presented in Table I.

TABLE I:CHARACTERISTICS SAMPLE BASED ON MOTHER'S AGE, GESTATIONAL AGE, BMI AND PARITY

Characteristic	HIV with ART 6 months	Without HIV	P-value
Maternal age	28 (7)	25 (8)	0.052 [a]
Gestational age	38 (1)	38 (2)	0.598 [a]
BMI	1 (1)	1 (2)	0.208 [a]
Parity	20.6 (3.07)	21.8 (2.49)	0.137 [a]

The median age of pregnant women at term was higher in mothers with HIV (+) on ART, namely 28 with an Inter Quartile Range (IQR) of 7 years compared to HIV (-) mothers. ie 25 with an IQR of 8 years. The median gestational age in the HIV (+) group on ART was 38 weeks with an IQR of 1 week, while in the HIV (-) group it was 38 weeks with an IQR of 2 weeks. Median parity in the HIV (+) group on ART was 1 with an IQR of 1, and in the HIV (-) group it was 1 with an IOR 2. Body mass index (BMI) was slightly higher in HIV mothers (-) namely 21.8 with an IQR of 2.49 kg/m 2 while in HIV (+) mothers on ART it is 20.6 with an IQR of 3.07 kg/m^2.

TABLE II: DISTRIBUTION OF CASPASE-3 EXPRESSION IN HIV (+) TERM PREGNANT WOMEN WITH ART TREATMENT ≥6 MONTHS AND PREGNANT WOMEN WITHOUT HIV

Group	Caspase 3 Expression		RP	95% CI	p value
	Tall	Low			
pregnant at term HIV (+) on ART ≥6 months	15 (75%)	5 (25%)			
pregnant at term HIV (-)	5 (25%)	15 (75%)	3,000	1.348 – 6.678	0.002

Normality test Caspase-3. expression data with the Saphiro-wilk test showed normal distribution (p> 0.05) and the homogeneity test data with Levene test showed Caspase-3 expression data homogeneous (p>0.05). Bivariate test was carried out by parametric test using independent t-test data is available in the appendix). The results showed that the mean expression of Caspase-3 was significantly higher in the placenta of HIV (+) pregnant women with ART. Treatment which is 162.62 (±36.83) compared to HIV pregnant women (-) which is 105.83 (±28.96).

N cut off value of expression Caspase-3 pregnant women at term HIV (+) with ART treatment 6 months and HIV (-) term pregnant women were obtained using receiver operating characteristics (ROC) curves (data available in appendix). The area under curve (AUC) value was 0.910 (95% CI = 0.824–0.996).

Cut-off value The selected Caspase-3 was 127.05 with a sensitivity of 75% and a specificity of 75%. The expression of Caspase-3 was then classified into high (≥127.05) and low (<127.05).

The chi-square test showed that the proportion of high Caspase-3 expression was significantly higher in the HIV (+) group on ART than in the non-HIV group (-) (75% vs 25%; p=0.002). Prevalence ratio value (RP) obtained >1 so ART therapy is a risk factor for high expression of C aspase-3 in pregnant women with HIV. It can be concluded that pregnant women infected with HIV and receiving antiretroviral therapy for 6 months had a 3 times greater chance of prevalence of high Caspase-3 expression than pregnant women who are not infected with HIV (Confidence Interval (CI) 95% = 1.348 – 6.678). This difference in the proportion of C aspase-3 expression based on HIV status is shown in Table II.

With 6 months ARV treatment, apoptosis of CD4 cells that occurs due to the bystander effect of apoptosis in HIV patients with untreated viral infection can be ruled out. This is considered to be comparable to the condition in patients without HIV infection. Due to the considerations above, the researchers took samples of term pregnant women without HIV infection as a group without risk factors [1], [8], [9].

Several studies have shown poor outcomes in pregnant HIV-infected patients with 6 months of ART treatment. Apoptosis that occurs in these patients is caused by the effect of NRTI drugs on DNA polymerase gamma (pol-γ). In vitro studies have shown that placenta exposed to ART accelerates ROS production, impaired mitochondrial function, and caspase-mediated cell death. These changes lead to degenerative changes in the placental villi in pregnant women treated with ART [10]-[12].

In this study, the median age of the mother on the patient HIV pregnant with ART treatment for 6 months is 2 8 (25 – 41) years while in patients without HIV it is 25 (21-41) years. These results are in accordance with the prevalence of cases at Sanglah Hospital, where most (68.86%) pregnant women who participated in the HIV Prevention of Mother to Child Transmission (PMTCT) program in the period 2005-2014 were aged 20-29 years [13].

This study showed no difference in gestational age between the two groups. The median gestational age in both groups was 38 (37-39) weeks. This result is similar to a previous study which showed that the gestational age at delivery in women with HIV was 37.5 (32.2-41.2) weeks, slightly lower than that of women without HIV, which was 38.6 (38.3-40,). 3) weeks [14].

The median parity in both groups was 1 (0-4). This is in accordance with previous research at Sanglah Hospital which found that the majority of HIV patients had parity 1 (42.85%) and 0 (24.79%) [13]. A study of 45 women with and without HIV each showed that the majority (75.6%) of the sample were multigravida and there was no difference in parity between the two groups [15]. Parity in HIV women is related to knowledge about their disease. There was a decrease in the percentage of mothers with parity 0 to parity 1 by 65% after knowing their HIV status. This is related to the fear of transmitting HIV to children, pregnancy will affect their health and financial limitations. So far, ART treatment has given hope to HIV-infected women by reducing the fear of death and transmitting HIV to the baby, thereby contributing to increased pregnancies in women with HIV [16].

Caspase-3 is one of the aspartate-specific proteases that play a role in the apoptosis of the caspase - dependent pathway. This pathway is triggered by receptor mediators from the extrinsic pathway through death receptor activation or intrinsically through mitochondrial metabolic failure [17], [18].

This study showed the mean expression of C aspase-3 in pregnant women with HIV infection with ART treatment for 6 months was 162.62 (±36.83), higher than mothers without HIV, which was 105.83 (±28.96). Receiver operating characteristics (ROC) curve gets value the cut-off of C aspase-3 expression was 127.05 pg/ml with 75% sensitivity and 75% specificity. The area under curve (AUC) value is 0.910 (95% CI = 0.824–0.996). The expression of C aspase-3 was then classified into high (≥127.05) and low (<127.05). The results of the bivariate test with chi-square test showed that the proportion of high Caspase-3 expression was significantly higher in the group of pregnant women with HIV infection and ART treatment 6 months. Pregnant women infected with HIV and on ART for 6 months had a 3 times higher chance of expressing C aspase-3 than pregnant women who were not infected with HIV.

Caspase-3 is the active form of procaspase-3 and is considered the most important caspase executor. Caspase-3 is activated by caspase initiators (C aspase-8, C aspase-9, C aspase-10) which then activate caspase-activated DNAse (CAD) endonucleases. In proliferating cells, CAD forms a

complex with its inhibitors, known as r inhibitors caspase-activated DNase (ICAD). In contrast, in apoptotic cells, activated C aspase-3 separates ICAD so that remaining CAD can disrupt chromosomal DNA in the nucleus, causing chromatin condensation. These components also trigger cytoskeletal regulation and separate cells to form apoptotic bodies. This process then results in phagocytosis [20].

Studies in pregnant patients with HIV infection receiving antiretroviral therapy have shown that viral proteins, including Vpr, are virtually undetectable [20]. Measurement of Caspase-3 /β-actin levels in neonates from HIV-infected pregnancies receiving ART showed that the results were not significantly different from controls (0.48±0.14 versus 0.47±0.17). This has led to the notion that the increased apoptosis observed in HIV-infected pregnancies is more likely to be mediated by ART use and duration of exposure than by virus. However, small amounts of Vpr or other viral proteins can exert a toxic effect [20].

The effect of NRTI group ART on DNA polymerase gamma is thought to trigger mtDNA depletion. If mtDNA decreases to a critical level, it can cause energy insufficiency and cell dysfunction. Several in vivo studies have found that giving zidovudine causes mitochondrial toxicity which is characterized by a decrease in mtDNA and abnormalities of the OXPHOS enzyme

Several pathophysiological mechanisms have been proposed to explain the occurrence of cell apoptosis that triggers morbidity in HIV-infected mothers. Mitochondria are specifically inherited from maternal ova and early ART exposure to ova and mitochondria will also affect fetal development. Previous studies have also shown that oocytes from HIV-infected women receiving antiretroviral therapy have decreased mtDNA, which is also a marker of cell apoptosis [7]. Based on the discussion above, it can be concluded that mothers pregnant term with infection human immunodeficiency virus that gets antiretroviral therapy has expression high levels of Caspase-3 in the placenta.

III. Conclusion

Based on the above discussion, it can be concluded that term pregnant women with human immunodeficiency virus infection who received antiretroviral therapy had high caspase-3 expression in the placenta.

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